STUDIES ON THE EFFECTS OF IRRADIATION OF CELLULAR PARTICULATES. V. ACCELERATION OF RECOVERY OF PHOSPHORYLATION BY POLYANIONS [1]

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While the major part of the effect of cell-free extracts of spleen on the recovery of irradiated animals may be attributed to cellular contamination (Koller, Davies and Doak, 1961), there are several studies which suggest that such extracts may be effective in accelerating the recovery of irradiated animals (Ellinger, 1956, 1957; Panjevac, Ristic and Kanazir, 1958). It must be clear that, regardless of the source of the cells, regeneration of radiation-damaged areas involves a growth process for which energy must be supplied. Since the great bulk of energy available for growth in higher organisms is supplied by the process of oxidative phosphorylation, and since it has been demonstrated that oxidative phosphorylation is uncoupled largely as an indirect effect of exposure to radiation (Benjamin and Yost, 1960; Yost, Glickman and Beck, 1964), it seemed logical to investigate the effects of various protective agents on the phosphorylating mechanism. The common characteristic of cell-free extracts is that the effective agent appears to be a compound of high molecular weight, probably a nucleic acid or nucleoprotein (Cole and Ellis, 1954). The finding of Allfrey and Mirsky (1958) that polyanions are capable of restoring phosphorylative activity in isolated nuclei suggested the possibility of a dependence upon polyanions of all phosphorylating mechanisms. Consequently, tests of various polyelectrolytes were designed to see what effect their injection into irradiated animals might have.

Since preliminary experiments indicated that the phosphorylating mechanism was protected by cell-free spleen extract, it seemed possible to extend the investigation to a consideration of the relationship of oxidative phosphorylation to recovery processes in general. If the process of oxidative phosphorylation be central to the regeneration process, restoration of phosphorylation at an appropriate time should make possible higher survival in irradiated organisms. Therefore, a secondary consequence of the initial investigation was the testing of some of these compounds for their ability to promote survival in irradiated animals, both as a study of the general problem of protection against radiation damage and as a test of the hypothesis that damage to the phosphorylating mechanism is causally related to the recovery process.

Materials and Methods

All experiments were carried out with male, albino rats of the Sprague-Dawley strain, weighing between 125 and 175 grams. The rats were irradiated without

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anesthetic in a small wire cage which permitted little freedom of motion. All radiation was delivered from a 175-curie Co^{60} source, filtered with one-half inch of Lucite, at an intensity of 65 r/min. All rats received 800 r total-body, the dose being calculated for a plane passing through the pituitary and spleen of the animal.

Rats were sacrificed by a sharp blow to the head, and the tissue for study was removed immediately. In the case of spleen tissue, two or three spleens were pooled and homogenized; for liver, a slice weighing approximately 2½ grams was removed from each rat; and in the case of the testis, four testes (two rats) were utilized for a single assay. The tissue was homogenized in cold isotonic sucrose containing 0.005 *M* disodium versenate. The mitochondria were isolated from the homogenate by differential centrifugation (Schneider, 1948), with the particulates collected at 9000 *g* and washed once. Mitochondria from control spleens and from liver, whether experimental or control, were resuspended in one ml. of sucrose solution. Mitochondria from irradiated spleen and from testis were resuspended in 0.5 ml. sucrose solution. One-half ml. of mitochondrial suspension was added to each Warburg flask for assay. The unequal dilutions of the different preparations were made necessary by the differences in yield of mitochrondria from the various organs and animals. These dilutions provide a method for getting reproducible results and provide the most conservative way of obtaining these results (Yost, Glickman and Beck, 1964).

The efficiency of respiratory energy conversion was measured using the P:O ratio. Oxygen uptake was measured in a Warburg respirometer at 25° C. Readings were taken for 20 to 30 minutes, after a five-minute equilibration period. Satisfactory P:O ratios were obtained from concentrated preparations, so long as 6 micro-atoms of oxygen were consumed within that period. Succinate was used as the substrate, and the incubation medium was the same as that described previously (Yost and Robson, 1959). Estimation of the remaining inorganic phosphorus was carried out by the method of Lowry and Lopez (Glick, 1949). All phosphate tests were run in duplicate.

For the preparation of the cell-free spleen extract, a slight modification of the procedure of Ellinger (1956) was employed. Sampling of spleens from animals weighing the same as those to be injected indicated an average spleen weight of 0.80 gram. Since it was desired to inject each rat with 2.5 ml. of extract containing the material from one spleen, the entire collection of removed spleens was weighed and then homogenized in a volume of cold 0.9% NaCl solution equivalent to 2.5 ml. per spleen. The homogenate was centrifuged for 30 minutes at 600 *g* to remove gross particulate matter (primarily nuclei and cell fragments). The supernatant fraction was filtered through several layers of filter paper in a Buechner funnel and then, under high vacuum, through a Selas bacteriological (0.3) filter. From this point on, sterile technique was followed. The resulting extract was transferred to vaccine bottles and kept cold until use (extracts were kept no longer than two days from the time of preparation). Samples were examined under the microscope for whole-cell counts. In all cases the extract was found to be devoid of whole spleen cells and of bacterial contamination. (As an added check, spleen preparations were spread on nutrient agar but no colony growth was observed.) Appropriate aliquots were withdrawn and injected intraperitoneally.

In the preparation of the extracts used to test the activity of various fractions of the homogenate, nuclei were separated from the rest of the material by centrifugation at 600 *g*. Prior to centrifugation the homogenized spleens were filtered through four layers of fine cheesecloth to remove whole cells and much of the cellular debris. This technique permitted the passage of many nuclei, which could be observed in the microscope. Precipitated nuclei were washed by resuspending in sucrose and recentrifugation. After washing, the resuspended nuclear pellet was allowed to stand for 30 minutes, during which time the nuclei settled to the bottom. The top 95% of the liquid was withdrawn and the remaining suspension was used to prepare an extract (by steps similar to those above) labeled the "nuclear" fraction. The supernatant fraction was derived from the several centrifugations made during the isolation of the nuclei. This collected supernatant was centrifuged at speeds slightly in excess of 600 *g*. The supernatant was decanted and then repeated centrifugations were made until no sediment was detected. The resulting clear, deep red solution was used as the "supernatant fraction." From such a procedure it is clear that the nuclear fraction is derived primarily from cell nuclei, although a certain amount of cytoplasmic material may

TABLE I

Effects of various cell-free spleen extracts on oxidative phosphorylation in spleen mitochondria from rats receiving 800 r total-body

Days after exposure	Non-injected	Saline	Whole homogenate	Supernatant	"Nuclear" fraction	No. runs
1	1.4	1.3	1.8	1.1	1.8	5
3	1.5	1.5	1.9	1.2	1.8	5
5	1.4	1.4	1.9	1.2	1.9	3
8	1.4	1.4	1.9	1.1	1.8	3
Control (ave.)	1.9	1.9	1.9	1.9	1.9	16

be carried along. In the case of the supernatant fraction, it is clear that the extract contains a large number of elements other than nuclei, including the mitochondria, etc. The sum of the two fractions would not contain all of the elements to be found in an extract of a complete homogenate of cells. In the repeated washings and centrifugations, a number of elements are lost. Thus, three rather different extracts were made: one from whole-cell homogenates, one from homogenates of isolated nuclei, and one from "homogenates" of cellular materials smaller than those precipitated in the nuclear fraction. These three extracts are identified in Table I as "whole homogenate," "nuclear fraction" and "supernatant."

Three classes of compounds were tested for their effect upon the phosphorylation mechanism: spleen extract, polycations and polyanions. Rats receiving the spleen extract were given injections of 2.5 ml.; in each case one set of control rats were injected with 2.5 ml. of 0.9% NaCl. The other compounds were injected in volumes ranging from 0.5 to 2.0 ml. In these latter cases, both irradiated and unirradiated control rats were injected with an equal volume of saline or water, depending upon the method of preparation of the experimental compounds. The polyanions used were polyethylene sulfonate, 5900 and 12,500 M.W. (Upjohn

Co.), desoxyribonucleic acid, standard and high polymer (Nutritional), and ribosenucleic acid, yeast (Nutritional). The polycations used were salmine (Nutritional) and lysozyme (Worthington). The lysozyme was inactivated by heat prior to injection. All injections were made intraperitoneally, in the anterior-abdominal region.

Survival tests were run for 30-day periods. In each test 90 rats were used, divided as follows: controls, 800 r whole-body, and 800 r whole-body injected with polyethylene sulfonate. Daily counts of the rats were kept and all tests were repeated three times.

Results

The design of the experiments and the method of presenting the data have been discussed previously in detail (Yost, Glickman and Beck, 1964). The following two paragraphs are intended to summarize the previous discussion and to facilitate interpretation of the data.

The data in the various tables are presented as average P:O ratio values for each set of experiments done at a particular condition, since the number of experiments reported in this paper is too great to permit the presentation of each experiment as a separate datum. In the case of the controls, the values for all runs have been averaged, since the variation for control animals is relatively small and repeated assays of this type have yielded control average values of 1.8 or 1.9 for livers and spleens, and 1.2 for testes. There is, of course, a certain amount of variation intrinsic to the P:O ratio determinations. Thus, one might expect, for an average P:O value of 1.9, to find a range of values in a set of individual experiments running from 1.7 to 2.2, or, for a value of 1.4, a range of values from 0.9 to 1.6. In each case, however, a clear difference exists between experimental and control animals. There is *never* an overlap of the values in individual experiments, so long as inactivation occurs. The consistency of these findings is remarkable, considering the internal variations to be expected in the system, and this suggests that it is perfectly permissible to present only the average values without presenting the range of each mean value. Percentage inactivations can be derived from the control values in each column of the data tables. As a check for the validity of this method, calculations were made on the basis of the average data and on the basis of the individual runs; in all cases the values were essentially the same. Thus, there is no distortion of the meaning of the data by the method of presentation.

Similarly, there is difficulty in the development of a test for the significance of differences in data of this type. Where the number of tests is sufficiently great (6 to 10 runs), standard deviation values of approximately 0.15 are obtained. Thus, to get highly significant results, it is necessary that the differences in P:O ratios between controls and experimentals be greater than 0.5. In Table I (and other tables as well) some lines of data can be demonstrated to be significantly different from one another on the basis of standard deviation tests, but most of the lines cannot. On the other hand, if the data for different days are summed and averaged, there are highly significant differences on the basis of standard deviation analysis. Furthermore, since the pattern remains unchanged and since experimental values at no time overlap control values (so long as an inactivation has occurred), it seemed permissible to lump the data for statistical

treatment by Chi-square analysis. When this is done the difference between the experimentals and their appropriate controls is highly significant ($P < 0.001$). Thus, the data in this paper clearly indicate that inactivation of oxidative phosphorylation occurs after exposure to radiation, and that the administration of several different compounds is capable of either removing or preventing this inactivation.

The data in Table I show that a cell-free extract of spleen tissue is capable of restoring oxidative phosphorylation in irradiated rats. The rats were injected shortly after exposure and assayed at various times after exposure. It can be seen that the "protective" effect of the extract is observable 24 hours after exposure. At this time, the effect of the irradiation is at its maximum in the control-irradiated animals (Yost, Glickman and Beck, 1964). Furthermore, the effect of the extract appears to be permanent; that is, it does not recede with time. Thus, it appears that cell-free spleen extracts are capable of making a permanent restoration of the phosphorylating mechanism in the surviving cells of the spleen. Furthermore, it is clear that the activity of this extract is limited to the nuclear fraction of the homogenate. Thus, whatever the protective agent may be, it appears to be localized in the nuclei of the spleen cells from which the extract is made. Neither saline nor saline extract of the non-nuclear fraction (designated "supernatant" in Table I) is capable of restoring the phosphorylating mechanism.

In an attempt to assess the general nature of the protective effect demonstrated by the data presented above, rats were injected with a cell-free spleen extract of the whole homogenate type immdiately after irradiation, and the phosphorylating ability of liver and testis mitochondria was assayed at various times after exposure (Table II). In liver and testis, maximum damage has been achieved by 48 hours after exposure, and therefore, two-day intervals were taken as a test of the regeneration of the system. Since after 10 days liver phosphorylation has recovered without treatment, only the first 8 days post-irradiation were used for assay. In the case of the testis, assays were made until the 14th day, after which period normal recovery has been effected. It can be seen that the extract is capable of restoring the phosphorylating mechanism on a permanent basis in both the liver and testis mitochondria. Therefore, the effect of the spleen extract is not specifically upon spleen cells; rather, it is a general effect of some component derived from the spleen cells. In both cases, there was no significant effect of the saline extraction medium on the recovery of the phosphorylating mechanism.

On the basis of the observations of Allfrey and Mirsky (1958) that polyanions restore uncoupled nuclear phosphorylation, it was decided to try a synthetic polyanion to see if it would replace the spleen extract in the recovery process. The data presented in Table III indicate that polyethylene sulfonate is just as effective as the spleen extract in causing the rapid and permanent recovery of the phosphorylating mechanism in irradiated rats. In this case, as in the case of the spleen extract, injections of polyethylene sulfonate were made immediately following irradiation, and assays were made at various times after exposure. The effect of the polyethylene sulfonate has been achieved by 24 hours after exposure and continues throughout the assay period. Furthermore, both the 12,500 MW

TABLE II

Effect of cell-free spleen extract on oxidative phosphorylation in liver and testis mitochondria from rats receiving 800 r total-body

Days after exposure	Liver			Testis		
	Sham-injected	Injected	No. runs	Sham-injected	Injected	No. runs
2	1.2	1.8	6	0.8	1.2	6
4	1.3	1.8	6	0.8	1.1	6
6	1.5	1.8	6	0.9	1.2	6
8	1.5	1.8	6	—	—	—
10	—	—	—	0.8	1.2	6
14	—	—	—	1.0	1.2	6
Control (ave.)	1.8	1.8	24	1.2	1.2	30

and 5900 MW compounds are satisfactory for restoring the phosphorylating mechanism. Thus, a molecular weight of no more than 6000 is necessary to effect the observed change. To demonstrate the significance of the protective effect of PES, the data from Table III were used to make a Chi-square analysis of the null hypothesis that there would be no significant difference in the distribution of either the sham-injected or injected values above or below the minimum normal value of 1.8. The Chi-square values indicate that the distribution of the sham-injected values below 1.8 and the injected values above 1.8 is highly significant, with the probability of occurrence by chance less than 0.001. This rejection of the null hypothesis clearly shows that PES is responsible for the normal values obtained in the experimental rats.

Having demonstrated that the polyanion, PES, was capable of restoring oxidative phosphorylation in irradiated rats, it seemed logical to test different polyanions and polycations. Table IV presents the data obtained with three polyanions (PES, RNA and DNA) and two polycations (salmine and lysozyme). The assays were made on liver mitochondria from a single rat liver, to avoid

TABLE III

Effect of polyethylene sulfonate on phosphorylation in spleen mitochondria from rats receiving 800 r total-body

Days after exposure	12,500 MW			5900 MW		
	Sham-injected	Injected	No. runs	Sham-injected	Injected	No. runs
1	1.6	1.9	5	1.4	1.9	5
2	1.5	1.9	3	—	—	—
3	1.4	1.9	3	—	—	—
8	1.5	1.9	5	1.4	1.8	5
14	1.5	1.9	7	—	—	—
Control (ave.)	1.9	1.9	23	1.4	1.9	10

pooling two or three rats as is necessary in analyses with spleen mitochondrial preparations. It can be seen that PES restores the liver phosphorylation, as it did the spleen phosphorylation, and thus the system seems adequate for comparisons of other polyanions and polycations. The data indicate that polyanions restore oxidative phosphorylation and that polycations are incapable of making the restoration. In the case of highly polymerized DNA (DNA-2), no effect on phosphorylation was observed. Unfortunately, with highly polymerized DNA preparations, it is hard to be sure that the injection is effective since the viscosity

TABLE IV

Effect of various polyelectrolytes on oxidative phosphorylation in liver mitochondria from rats receiving 800 r total-body

	12 hours post-irrad.	2 days post-irrad.	8 days post-irrad.
Sham-injected	1.5	1.6	1.6
PES	2.0	1.9	1.9
No. runs	6	6	4
Sham-injected	—	1.5	1.5
RNA	—	1.9	1.8
No. runs	—	10	3
Sham-injected	1.3	1.5	1.5
DNA-1*	1.8	1.9	1.9
No. runs	3	5	3
Sham-injected	—	1.6	—
DNA-2*	—	1.6	—
No. runs	—	4	—
Sham-injected	1.2	1.2	1.4
Salmine	1.2	1.2	1.4
No. runs	3	4	3
Sham-injected	—	1.2	—
Lysozyme	—	1.3	—
No. runs	—	3	—
Controls (ave.)	1.9	1.9	1.8
No. runs	12	21	13

* DNA-1 is the usual commercial grade; DNA-2 is highly polymerized.

of the solution makes intraperitoneal injections extremely difficult. Furthermore, when the molecular weight is raised to a very high level, it may be possible that permeability problems become overpowering. This might be the reason for the failure for the polycation, lysozyme, to have any effect on the phosphorylating mechanism. On the other hand, it seems unlikely that salmine would be incapable of penetrating, and, therefore, these data can be taken as presumptive evidence that the polycations have a different effect than the polyanions on the phosphorylating mechanism of irradiated rats. It is certainly clear that lower

TABLE V

Effect of polyethylene sulfonate, injected pre- and post-exposure to 800 r total-body, on spleen phosphorylation

Days after exposure	24 hours post-ex.			5 hours pre-ex.		
	Sham-injected	Injected	No. runs	Sham-injected	Injected	No. runs
2	1.6	2.0	5	1.4	1.5	3
8	1.5	1.8	4	1.5	1.5	3
Control (ave.)	1.9	1.9	9	1.9	1.9	6

molecular weight polyanions, such as RNA, DNA and PES, are capable of restoring the phosphorylating mechanism on a permanent basis.

The data in Table V show the effect of polyethylene sulfonate when injected either prior to or after exposure to radiation. Although results with spleen are presented here, similar experiments have been done with liver mitochondrial preparations and the data are essentially the same. The most striking aspect of these results is that PES can be injected 24 hours after the exposure to radiation and still result in a complete recovery of the phosphorylating mechanism. On the other hand, pre-irradiation injection of PES appears to have no effect. Thus, in the five hours prior to radiation, either the PES is incorporated (or excreted) by the rat and is no longer available to do whatever it does in the restoration process, or the exposure to radiation alters the PES in such a way that it is no longer effective.

Having demonstrated that the PES can restore the phosphorylating mechanism, it seemed wise to test the effect of this compound upon survival. Table VI presents the data derived from survival studies performed under different conditions: two different doses of radiation, and two methods of injection of the PES. It can be seen that there is no significant difference in the survival of animals injected

TABLE VI

Survival of rats receiving total-body irradiation and polyethylene sulfonate

Treatment	Initial	Final	% Survival
Control	120	120	100
400 r	120	98	82
400 r + PES*	120	97	81
Control	120	118	98
800 r	120	38	32
800 r + PES*	123	38	31
Control	120	119	99
800 r	120	43	36
800 r + PES**	120	50	42

* 20 mg. in 2 ml.
** 20 mg. in 0.5 ml.

with PES over those which were uninjected, and, thus, it would appear that PES is capable of influencing the regeneration of the phosphorylating mechanism without influencing survival.

Discussion

The results presented in this paper show that a non-biological polyanion, PES, is capable of restoring oxidative phosphorylation in irradiated rats and that the effect of this compound is essentially the same as that of RNA, DNA or cell-free spleen extracts. Thus, the results obtained here are quite similar to those obtained by Allfrey and Mirsky (1958) with nuclear phosphorylation, indicating that either a polymer of DNA or a similarly charged polymer (PES) could restore an uncoupled phosphorylating mechanism. Apparently the action of PES in irradiated rats is the same as, or at least very similar to, the action of the nucleoprotein extracted from spleen and used by Cole and Ellis (1954) and by Ellinger (1957).

Since the PES can be injected as much as 24 hours after exposure to radiation and still restore the mechanism, it is clear that the polyanions are not acting as protective agents in the usual sense. That is to say, their function seems to be "restorative," agents which act on the regeneration process. Since at 24 hours both the phosphorylating mechanism and the lymphocyte population of the spleen are at their lowest levels (Yost, Glickman and Beck, 1964), it is clear that the radiation damage, through whatever channel it may be effected, has already been achieved. From this point on, only the regeneration mechanism can be affected by the various agents. Thus, the effect of polyanions must be attributed to their ability to restore the phosphorylating chain in some way, rather than any effect they might have in removing "toxic substances" produced by the radiation.

The function of polyanions in restoring the phosphorylation process is obscure; however, a certain parallel may be drawn betwen the studies presented here and other work on the normal mechanism of coupling in isolated phosphorylating particles. It has been demonstrated several times that mitochondrial RNA has a role in the normal coupling of phosphorylation and oxidation (Yost and Robson, 1959; Hanson, 1959; Linnane and Titchener, 1960). Pinchot (1959) has shown that an RNA "cofactor" is required to bind a soluble, heat-labile fraction essential for phosphorylation to the cytochrome fraction serving as an electron transport system. The binding is nonspecific, since it is possible to replace the extracted "cofactor" with synthetic polynucleotides. Thus, it would appear that the function of a polyanion might be the binding of phosphorylating enzymes to the electron transport site (most likely, by a ligand with the aid of Mg^{++}).

If the preceding hypothesis for the function of polyanions be correct, the effect of the polycations might only be detected at later dates. If the polycations were incorporated into the system in place of polyanions, they might stabilize the uncoupling by preventing the entrance of new RNA polymers to the phosphorylating site and thus prevent "wound healing." An examination of the long-term effect of polycations on uncoupled preparations is in process, but the data do not permit any conclusions at the present time.

It is possible that the role of the polyanion is the removal of a naturally-occurring uncoupling agent released from damaged mitochondria, since it has been

demonstrated that the process of ageing results in structural changes in the mitochondria which release an uncoupler composed primarily of lipids and hemeproteins (frequently called mitochrome). This seems quite possible in view of the finding of Lehninger (1959) that the lipid uncoupling agents are probably released under the influence of hormones (such as thyroxin) and the finding of Wojtczak and Wojtczak (1960) that serum albumen protects mitochondrial phosphorylation by complexing with fatty acids. If such a mechanism were the correct explanation of the effect of the polyanions, it would be possible to duplicate the effect with *in vitro* experiments. Experiments were performed in which either DNA-1 or PES were added to isolated mitochondria. In neither case was it possible to obtain a restoration of the phosphorylating mechanism. In the case of PES, 20 mg. and 40 mg. were added to each Warburg flask, but no effect of the polyanion could be demonstrated. Thus, the function of the PES is not through the removal of an uncoupling agent in the mitochondrial preparation. Apparently it is necessary for the intact cell to aid in the incorporation of the polyanion to achieve the recoupling of phosphorylation.

The failure of PES to increase survival may be interpreted in three ways. In the first place, it may be taken as a direct confirmation of the cellular repopulation hypothesis, suggesting that nothing but the addition of cellular elements will increase the regeneration process in irradiated organisms (Koller, Davies, and Doak, 1961). Second, it may be taken to indicate that, in those organisms which are destined to survive in the population, enhancement of the recovery process by restoration of oxidative phosphorylation will only result in an increased *rate* of recovery and not in a significantly increased number of survivors. Unfortunately, the way the survival studies are generally conducted does not test this hypothesis. It is necessary to study the day-by-day events at a histological level to determine whether or not the injection of PES is capable of accelerating the rate of recovery. Such studies are now in progress. Finally, the data may indicate that the uncoupling of phosphorylation is only indirectly related to the regenerative process. The uncoupling may be a generalized stress response of the rat which has the net effect of accelerating the general metabolism to permit a more rapid synthesis of intermediate compounds necessary to "rebuild" damaged areas.

It may well be that if proper concentrations of PES are tried, increases in survival will be found. It must be remembered that certain cell-free spleen extracts increase both the regeneration of the phosphorylating mechanism (these data) and survival (Ellinger, 1957). Therefore, it is possible that the failure of the PES to increase survival is a concentration problem (or a molecular weight problem). Thus, before any final conclusions can be drawn about the value of polyanions in survival studies, it will be necessary to carry out extensive experiments on the survival of treated organisms. Such studies are beyond the scope of this laboratory.

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Summary

Male rats exposed to 800 r gamma radiation were injected post-irradiation with various polyanions and polycations and with a cell-free extract of rat spleen. Inactivation of oxidative phosphorylation by the irradiation was reversed by the spleen extract and by the polyanions, but not by the polycations. The data suggest that one of the functions (and possibly the only function) of cell-free extracts derived from spleen tissue is the restoration of oxidative phosphorylation in irradiated organisms. Since this effect can be duplicated by any of several commercially prepared polyanions, it seems unlikely that the spleen has in it a special agent responsible for effecting radiation protection. The data obtained with oxidative phosphorylation are in no way contradictory to the cellular replacement hypothesis of increased survival after exposure to radiation; they merely explain a possible mode of action of humoral agents which are known to have a limited effect on survival. Failure of PES to increase survival under the same conditions in which it recouples phosphorylation would seem to indicate that survival and regeneration of the phosphorylation mechanism are independent processes.

LITERATURE CITED

Allfrey, V. G., and A. E. Mirsky, 1958. Some effects of substituting the deoxyribonucleic acid of isolated cell nuclei with other polyelectrolytes. *Proc. Nat. Acad. Sci.*, **44**: 981–991.

Benjamin, T. L., and H. T. Yost, 1960. The mechanism of uncoupling of oxidative phosphorylation in rat spleen and liver mitochondria after whole-body irradiation. *Rad. Res.*, **12**: 613–625.

Cole, L. J., and M. E. Ellis, 1954. Studies on the chemical nature of the radiation protection factor in mouse spleen. I. Enzymatic inactivation by DNAases and trypsin. *Rad. Res.*, **1**: 347–357.

Ellinger, F., 1956. Further studies with cell-free extracts from mouse spleen on X-ray induced mortality. *Proc. Soc. Exp. Biol. Med.*, **92**: 670–673.

Ellinger, F., 1957. Protection of guinea pigs against radiation death by cell-free mouse spleen extracts. *Science*, **126**: 1179–1180.

Glick, D., 1949. Techniques of Histo- and Cytochemistry. Interscience Publishers, Inc., New York.

Hanson, J. B., 1959. The effect of ribonuclease on oxidative phosphorylation by mitochondria. *J. Biol. Chem.*, **234**: 1303–1306.

Koller, P. C., A. J. S. Davies and S. Doak, 1961. Radiation Chimeras. *In:* Advances in Cancer Research, A. Haddow and S. Weinhouse, Eds., Academic Press, New York. Vol. VI. Pp. 181–291.

Lehninger, A. L., 1959. An endogenous uncoupling and swelling agent in liver mitochondria and its enzymatic formation. *J. Biol. Chem.*, **234**: 2459–2464.

Linnane, A. W., and E. B. Titchener, 1960. Studies of the mechanism of oxidative phosphorylation. VI. A factor for coupled oxidation in the electron transport particle. *Biochem. et Biophys. Acta*, **39**: 469–478.

Panjevac, B., G. Ristic and D. Kanazir, 1958. Recovery from radiation injury by rats following administration of nucleic acids. *Second Internat. Conf. Peaceful Uses Atomic Energy*, **23**: 64–70.

Pinchot, G. B., 1959. A polynucleotide coenzyme of oxidative phosphorylation. III. Mechanism of action. *Biochem. Biophys. Res. Communs.*, **1**: 17.

Schneider, W., 1948. Intracellular distribution of enzymes. III. The oxidation of octanoic acid by rat liver fractions. *J. Biol. Chem.*, **176**: 259–266.

van Bekkum, D. W., 1957. The effects of X-rays on phosphorylation *in vivo*. *Biochim. et Biophys. Acta*, **25**: 487–492.

WOJTCZAK, L., AND A. B. WOJTCZAK, 1960. Uncoupling of oxidative phosphorylation and inhibition of ATP-P_i exchange by a substance from insect mitochondria. *Biochim. et Biophys. Acta,* **39**: 277–286.

YOST, H. T., R. M. GLICKMAN AND L. H. BECK, 1964. Studies on the effects of irradiation of cellular particulates. IV. The time sequence of phosphorylation changes *in vivo*. *Biol. Bull.*, **127**: 173–185.

YOST, H. T., AND H. H. ROBSON, 1959. Studies on the effects of irradiation of cellular particulates. III. The effect of combined radiation treatments on phosphorylation. *Biol. Bull.*, **116**: 498–506.